

18 January 2005

Whatman
Exhibit 5

Christopher Jones
84 Fowlmere Road
Heydon
Herts SG8 8PU

9 326 1740
9 326 1741
DDI: +44 (0) 208
Website: www.whatman.com

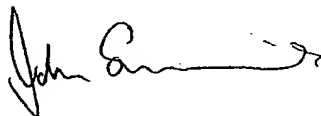
Dear Chris,

You will doubtless recall that you are a co-inventor (together with Neil Butt) in respect of an invention relating to "Separation of plasmid and genomic DNA using butanol" where a series of patent applications was filed in the name of Whatman Bioscience Limited.

For the US application (No 09/856336 - copy enclosed) we are required by the US Patent Office to file a Supplemental Declaration and Power of Attorney which requires your signature.

This is enclosed. I would be very grateful if you would execute this and return it to us as soon as possible (preferably by Registered Post, the cost of which we will be happy to refund).

Yours sincerely,



JOHN SIMMONDS
Company Secretary



SUPPLEMENTAL DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed at 201) below or an original, first and joint inventor (if plural names are listed at 201-206 below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NUCLEIC ACID ISOLATION

which is described and claimed in:

- ☐ the specification attached hereto.
- ☒ the specification in U.S. Application Serial Number 09/856,336, filed on May 17, 2001.
- ☐ the specification in PCT international application Number, _____, filed on _____; and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign/PCT Applications and Any Priority Claims Under 35 U.S.C. §119:			
Application No.	Filing Date	Country	Priority Claimed Under 35 U.S.C. §119?
9825215.8	17 November 1998	United Kingdom	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first

paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Applications or PCT International Applications Designating the U.S.-Benefit Under 35 U.S.C. §120					
U.S. Applications		Status (Check One)			
Application Serial No.	U.S. Filing Date	Patented	Pending	Abandoned	
PCT Applications Designating the U.S.					
Application No.	Filing Date	U.S. Serial No. Assigned			
PCT/GB99/ 03830	17 November 1999				

**CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. §119(e))**

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Applicant	Provisional Application Number	Filing Date

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

David G. Conlin (Reg. No. 27,026)
George W. Neuner (Reg. No. 26,964)
Linda M. Buckley (Reg. No. 31,003)
Peter J. Manus (Reg. No. 26,766)
Peter F. Corless (Reg. No. 33,860)

Cara Z. Lowen (Reg. No. 38,227)
William J. Daley, Jr. (Reg. No. 35,487)
David A. Tucker (Reg. No. 27,840)
Robert L. Buchanan (Reg. No. 40,927)
Christine C. O'Day (Reg. No. 38,256)

Lisa Swiszc Hazzard (Reg. No. 44,368)
George W. Hartnell, III (Reg. No. 42,639)
Steven M. Jensen (Reg. No. 42,693)
Kathryn A. Piffat (Reg. No. 34,901)

SEND CORRESPONDENCE TO:

DIRECT TELEPHONE CALLS TO:

David G. Conlin Intellectual Property Practice Group Edwards & Angell, LLP P.O. Box 55874 Boston, Massachusetts 02205	David G. Conlin (617) 439-4444
---	-----------------------------------

201	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE
		Butt	Neil	James
		Cambridge CB2 2LY	United Kingdom	British
		15 Petworth Street	Cambridge CB2 2LY	United Kingdom
202	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE
		Jones	Christopher	Peter
		Hertfordshire SG8 8PU	United Kingdom	British
		84 Fowlmere Road, Heydon	Hertfordshire SG8 8PU	United Kingdom
203	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE
204	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE
205	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor 201	Signature of Inventor 202 ✓
Date:	Date: ✗
Signature of Inventor 203	Signature of Inventor 204
Date:	Date:
Signature of Inventor 205	Signature of Inventor 206
Date:	Date:

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 May 2000 (25.05.2000)

PCT

(10) International Publication Number
WO 00/29563 A1

(51) International Patent Classification: C12N 15/10

(21) International Application Number: PCT/GB99/03830

(22) International Filing Date:
17 November 1999 (17.11.1999)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9825215.8 17 November 1998 (17.11.1998) GB

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): CAMBRIDGE MOLECULAR TECHNOLOGIES LIMITED [GB/GB]; Unit 3, Cambridge Techno Park, Newmarket Road, Cambridge CB5 8PB (GB).

Published:
— With international search report.

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): BUTT, Neil, James [GB/GB]; 15 Petworth Street, Cambridge CB2 2LY (GB). JONES, Christopher, Peter [GB/GB]; 84 Fowlmere Road, Heydon, Herts SG8 8PU (GB).

(48) Date of publication of this corrected version:
1 March 2001

(15) Information about Correction:
see PCT Gazette No. 09/2001 of 1 March 2001, Section II

(74) Agents: DANIELS, Jeffrey, Nicholas et al.; Page White & Farrer, 54 Doughty Street, London WC1N 2LS (GB).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 00/29563 A1

(54) Title: NUCLEIC ACID ISOLATION

(57) Abstract: A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising extracting the plasmid DNA into a water-immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA and recovering the plasmid DNA from the organic phase.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/10	A1	(11) International Publication Number: WO 00/29563 (43) International Publication Date: 25 May 2000 (25.05.00)
---	----	--

(21) International Application Number: PCT/GB99/03830

(22) International Filing Date: 17 November 1999 (17.11.99)

(30) Priority Data:
9825215.8 17 November 1998 (17.11.98) GB

(71) Applicant (for all designated States except US): CAMBRIDGE
MOLECULAR TECHNOLOGIES LIMITED [GB/GB];
Unit 3, Cambridge Techno Park, Newmarket Road,
Cambridge CB5 8PB (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BUTT, Neil, James
[GB/GB]; 15 Petworth Street, Cambridge CB2 2LY (GB).
JOES, Christopher, Peter [GB/GB]; 84 Fowlmere Road,
Heydon, Herts SG8 8PU (GB).

(74) Agents: DANIELS, Jeffrey, Nicholas et al.; Page White &
Farrer, 54 Doughty Street, London WC1N 2LS (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG,
BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB,
GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,
KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG,
MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE,
SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ,
VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW,
SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY,
KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH,
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN,
GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

(54) Title: NUCLEIC ACID ISOLATION

(57) Abstract

A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising extracting the plasmid DNA into a water-immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA and recovering the plasmid DNA from the organic phase.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

NUCLEIC ACID ISOLATION

The present invention relates to a method for isolating nucleic acid, and particularly to a method for isolating plasmid DNA from a plasmid DNA-containing material.

Conventional procedures for the purification of nucleic acid, such as DNA, generally require multiple steps including lysis of source material followed by fractionation steps which may involve column chromatography. Where DNA manipulation is to be carried out, small scale DNA preparations are required routinely, often in large quantities for the purpose of screening DNA from the source cells. These processes are time consuming and labour intensive.

Various methods have been proposed in the purification of such DNA, including a precipitation method in EP-A-0376080, an ultrafiltration method in WO-A-87/07645 and EP-A-0517515 and cationic exchange resins in EP-A-0281390 and EP-A-0366438. A simplified method involving a filter, which is automatable, is disclosed in WO-A-95/02049.

Each of these methods suffers from a disadvantage that a series of steps is required and/or special apparatus is required to achieve sufficient purification of the plasmid DNA. A need therefore arises for a much simpler method involving readily-available apparatus and relatively inexpensive reagents. In a known approach for rapid purification of genomic DNA, RNA or protein, a mixture of phenol, chloroform and guanidine is used (Chomczynski, P. and Sacchi, N., 1987 Anal Biochem. 162: 156; Chomczynski, P., 1993 Biotechniques 15: 532) in which the DNA is extracted into an aqueous phase. This method is unsuitable for isolating plasmid DNA. Moreover, the use of phenol and chloroform is undesirable as these are toxic substances.

The present invention aims to overcome the disadvantages of the prior art and to provide a simplified method for isolating plasmid DNA.

Accordingly, the present invention provides a method for isolating plasmid DNA from DNA containing material which comprises plasmid DNA and genomic DNA, comprising:

(i) extracting the plasmid DNA into a water-immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA; optionally separating the organic and aqueous phases of step (i); and

(ii) recovering the plasmid DNA from the organic phase.

Accordingly, the present invention provides a "one step" method which is simple to perform and which requires no specialised laboratory apparatus. It is surprisingly found that this method is capable of extracting plasmid DNA to high purity and with particularly low or zero contamination from genomic DNA which might be present in the plasmid DNA-containing material. In a preferred arrangement, the organic solvent is capable of selectively supporting the plasmid DNA with the exclusion of genomic DNA present in the plasmid DNA-containing material.

The method of the present invention may be performed on a small routine laboratory scale working with solution volumes of microlitres or millilitres. Alternatively, the method may be scaled up even to pilot or industrial scale involving volumes of litres or greater.

In extraction step (i), the DNA-containing material is mixed with the reagents under conditions to denature the genomic DNA typically whereby the plasmid DNA is partitioned into an organic

phase and the genomic DNA is partitioned into an aqueous phase. Such conditions include basic conditions or elevated temperature. Suitable elevated temperatures are of at least 65°C and more preferably in the range 70 to 95°C for a time sufficient to denature the plasmid DNA such as from about 30s to about 10mins, preferably around five minutes. Incubation times longer than about 10 minutes at elevated temperature should not adversely affect the plasmid DNA but are undesirable for using the organic solvent. In a preferred arrangement, basic conditions are used in which a base is present. The base is typically a hydroxide such as an alkali metal hydroxide, preferably sodium hydroxide. The base is preferably present at a concentration in the range 100mM to 200mM. Incubation time is usually in the range from about 30s to about 10mins, preferably around five minutes. Excessive incubation under basic conditions can damage the plasmid DNA.

Without wishing to be bound by theory, it is thought that differential solubility between plasmid and genomic DNA under denaturing condition may result in plasmid DNA in an undenatured or reversibly denatured state partitioning into the organic phase. In contrast denatured genomic DNA partitions into the aqueous phase.

The organic solvent must be immiscible with the aqueous phase and preferably comprises an alcohol which may be aliphatic or aromatic and which may be linear or branched chain. The alcohol is preferably a C₃ to C₆ alcohol, more preferably a C₄ to C₆ alcohol and most preferably comprises a butanol such as N-butanol.

The chaotrope may be any normally-recognised chaotrope and is preferably selected from guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate and mixtures thereof. A preferred chaotrope is guanidine hydrochloride. Typically, the

chaotrope is present at a concentration in the range 0.7M to 1.2M, based on the combination of organic solvents, chaotrope water. The concentration of the chaotrope is preferably about 0.9M.

The amount of organic solvent is typically in the range from 20 to 70% based on the volume of the combination of organic solvent, chaotrope or water and is preferably in the range from 35 to 50%, more preferably around 42%.

The exact organic solvent, chaotrope, base and amounts thereof are readily determinable by routine experimentation. Each of these reagents may be mixed with the DNA-containing material in any order or may be premixed prior to addition to the plasmid-containing material. In a convenient arrangement, the organic solvent, chaotrope, base and water are combined to form an extraction mixture. In this arrangement, the extraction step (i) comprises mixing the extraction mixture with the DNA-containing material.

At laboratory scale, the step (ii) of separating the organic and aqueous phases may be conveniently carried out by allowing the phases to separate or encouraging separation on the basis of density by a short spin in a microcentrifuge. Typically, either the organic or aqueous phase is removed from the other prior to recovery step (iii). For example, the organic phase containing the plasmid DNA may be transferred from one container to another by pipette prior to recovery. On a larger scale, removal of one phase from the other could be performed by any conventional method including pumping or running off by gravity one of the two phases.

In one arrangement, recovery step (iii) includes precipitation of the plasmid DNA from the organic solvent. For example, the DNA-containing organic phase may be mixed with a precipitating

- 5 -

agent that can precipitate the plasmid DNA from the organic solvent and the precipitated plasmid DNA is separated from the solvent. The precipitated plasmid DNA may also be washed in a washing step. The precipitating agent may comprise an alcohol such as ethanol and may further comprise an acetate salt such as sodium acetate.

The DNA-containing material may comprise any known DNA-containing material such as a bacterial culture which may be lysed or unlysed.

In a further aspect, the present invention provides an extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises a water-immiscible organic solvent capable of supporting plasmid DNA, a chaotrope and water. The extraction mixture preferably further comprises a base.

The organic solvent, chaotrope, base and amounts thereof are typically those described above.

The present invention will now be described in further detail, by way of example only, with reference to the following Examples.

Example 1

General procedure

Bacterial culture (*E coli* containing pBluescript; 0.5ml) was spun down in an eppendorf tube using a microcentrifuge and the supernatant was discarded. The pellet was resuspended in TE buffer (tris(hydroxymethyl)aminomethane hydrochloride 10mM, EDTA 1mM; pH8.0; 200 μ l) to form a resuspended pellet containing both genomic and plasmid DNA. An extraction mixture was selected according to the Table below, mixed very well and 0.5ml thereof was added to the resuspended pellet and gently mixed. The eppendorf containing the mixture was spun in a microcentrifuge

for 30 seconds to yield two phases; an upper organic phase and a lower aqueous phase. The organic phase was removed carefully to a fresh eppendorf tube avoiding any contaminating debris. Following measurement of the volume of the removed organic phase, sodium acetate (0.1vols; 3M) and ethanol were added (2vols) to precipitate the plasmid DNA. The eppendorf was spun in a microcentrifuge for 20 minutes and the ethanol supernatant removed. The pellet was rinsed with fresh ethanol (70%; 200 μ l) and spun for 5 minutes. The ethanol was removed and the pellet dried and resuspended in water (20 μ l). The resultant plasmid-containing DNA solution could then be assayed by visualisation on an agarose gel and the amount of DNA determined quantitatively by spectrophotometry or by fluorescence.

Table of Extraction Mixtures Tested

<u>CHAOTROPE</u>	<u>NaOH</u>	<u>SOLVENT</u>	<u>PLASMID DNA RECOVERY</u>
GuSCN 0.9M	150mM	N-Butanol 42%	Poor
GuSCN 0.9M	90mM	N-Butanol 42%	Poor
GuSCN 0.9M	200mM	N-Butanol 42%	Poor
GuSCN 0.9M	90mM	N-Butanol 20%	Poor
GuSCN 0.9M	150mM	N-Butanol 20%	Poor
GuSCN 0.9M	200mM	N-Butanol 20%	Good
GuSCN 0.9M	90mM	N-Butanol 70%	No
GuSCN 0.9M	150mM	N-Butanol 70%	No
GuSCN 0.9M	200mM	N-Butanol 70%	No
GuSCN 0.9M	90mM	N-Butanol 42%	Good
GuHCl 0.9M	150mM	N-Butanol 42%	Good
GuHCl 0.9M	200mM	N-Butanol 42%	Good
GuHCl 0.9M	90mM	N-Butanol 20%	OK
GuHCl 0.9M	150mM	N-Butanol 20%	OK
GuHCl 0.9M	200mM	N-Butanol 20%	Poor
GuHCl 0.9M	90mM	N-Butanol 70%	OK
GuHCl 0.9M	150mM	N-Butanol 70%	OK
GuHCl 0.9M	200mM	N-Butanol 70%	Poor
GuHCl 0.9M	90mM	2 methyl propanol 20%	Poor
GuHCl 0.9M	150mM	2 methyl propanol 20%	Poor
GuHCl 0.9M	200mM	2 methyl propanol 20%	Poor
GuHCl 0.9M	90mM	2 methyl propanol 70%	No
GuHCl 0.9M	150mM	2 methyl propanol 70%	No
GuHCl 0.9M	200mM	2 methyl propanol 70%	No
GuHCl 0.9M	90mM	2 methyl propanol 42%	Poor
GuHCl 0.9M	150mM	2 methyl propanol 42%	OK
GuHCl 0.9M	200mM	2 methyl propanol 42%	OK

- 7 -

GuHCl 0.9M	90mM	Butan-2-ol 42%	Poor
GuHCl 0.9M	150mM	Butan-2-ol 42%	OK
GuHCl 0.9M	200mM	Butan-2-ol 42%	Good
GuHCl 0.9M	90mM	Butan-2-ol 20%	Poor
GuHCl 0.9M	150mM	Butan-2-ol 20%	Poor
GuHCl 0.9M	200mM	Butan-2-ol 20%	Poor
Na Perchlorate 0.9M	90mM	N-Butanol 42%	Poor
Na Perchlorate 0.9M	150mM	N-Butanol 42%	Poor
Na Perchlorate 0.9M	200mM	N-Butanol 42%	Poor
Na Perchlorate 0.9M	90mM	N-Butanol 70%	Poor
Na Perchlorate 0.9M	150mM	N-Butanol 70%	Poor
Na Perchlorate 0.9M	200mM	N-Butanol 70%	Poor
Na Perchlorate 0.9M	90mM	N-Butanol 20%	Poor
Na Perchlorate 0.9M	150mM	N-Butanol 20%	Poor
Na Perchlorate 0.9M	200mM	N-Butanol 20%	OK
Na Perchlorate 0.9M	200mM	2 methyl propanol 20%	OK
Na Perchlorate 0.9M	90mM	2 methyl propanol 70%	Poor
Na Perchlorate 0.9M	150mM	2 methyl propanol 70%	Poor
Na Perchlorate 0.9M	200mM	2 methyl propanol 70%	Poor
Na Perchlorate 0.9M	90mM	Butan-2-ol 42%	Poor
Na Perchlorate 0.9M	150mM	Butan-2-ol 42%	OK
Na Perchlorate 0.9M	200mM	Butan-2-ol 42%	No
Na Perchlorate 0.9M	90mM	Butan-2-ol 20%	No
Na Perchlorate 0.9M	150mM	Butan-2-ol 20%	No
Na Perchlorate 0.9M	200mM	Butan-2-ol 20%	No

Good Approximately 1µg DNA recovery
 OK Approximately 200ng DNA recovery
 Poor Just visible on agarose gel electrophoresis

It may be concluded from these results that each recognised chaotrope works and that the guanidine hydrochloride is preferred over the guanidine thiocyanate which is, in turn, preferred over sodium perchlorate in terms of DNA recovery. As to solvents, butanol was found to work best whereas pentanol gave only poor DNA recovery. Ethanol and isopropanol were found not to be water-immiscible. Of the butanols, N-butanol was found to be better than either butan-2-ol or 2 methyl propanol.

Whilst TE was used as the resuspension buffer in the procedure, water could also be used, as well as other resuspension buffers.

Example 2

General procedure for extraction using heat instead of alkaline pH

Bacterial culture (*E coli* containing pBluescript; 0.5ml) was spun down in an eppendorf tube using a microcentrifuge and the supernatant was discarded. The pellet was resuspended in TE buffer (tris(hydroxymethyl)aminomethane hydrochloride 10mM, EDTA 1mM; pH8.0; 200 μ l) to form a resuspended pellet containing both genomic and plasmid DNA. An extraction mixture was selected according to the Table below, mixed very well and 0.5ml was added to resuspended pellet and gently mixed. The eppendorf tube was then placed in a hot water bath at a temperature in the range 70 to 95°C for five minutes and the contents frequently mixed. Care was taken with the lid of the eppendorf tube because of solvent expansion in the tube. The tube was then rapidly cooled on ice for three minutes, which had the effect of separating the plasmid and genomic DNA. The eppendorf containing the mixture was spun in a microcentrifuge for 30 seconds to yield two phases; an upper organic phase and a lower aqueous phase. The organic phase was removed carefully to a fresh eppendorf tube avoiding any contaminating debris. Following measurement of the volume of the removed organic phase, sodium acetate (0.1vols; 3M) and ethanol were added (2vols) to precipitate the plasmid DNA. The eppendorf was spun in a microcentrifuge for 20 minutes and the ethanol supernatant removed. The pellet was rinsed with fresh ethanol (70%; 200 μ l) and spun for 5 minutes. The ethanol was removed and the pellet dried and resuspended in water (20 μ l). The resultant plasmid-containing DNA solution could then be assayed by visualisation on an agarose gel and the amount of DNA determined quantitatively by spectrophotometry or fluorescence.

Results comparable to those of Example 1 were obtained although yields were slightly lower and minor contamination with genomic DNA was observed.

CLAIMS:

1. A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising:

(i) extracting the plasmid DNA into a water-immiscible organic solvent capable of supporting plasmid DNA, by mixing the material with the organic solvent, a chaotrope and water under conditions to denature the genomic DNA; and

(ii) recovering the plasmid DNA from the organic phase.

2. A method as claimed in claim 1, wherein the organic solvent is capable of selectively supporting the plasmid DNA to the exclusion of denatured genomic DNA.

3. A method as claimed in claim 1 or claim 2, wherein the conditions to denature the plasmid DNA comprise basic conditions or a temperature of at least 65°C.

4. The method as claimed in claim 3, wherein the conditions to denature the plasmid DNA comprise basic conditions in which a base is present.

5. A method as claimed in claim 4, wherein the organic solvent comprises a C₃ to C₆ alcohol.

6. A method as claimed in claim 5, wherein the C₃ to C₆ alcohol comprises butanol.

7. A method as claimed in claim 6, wherein the butanol comprises n-butanol.

8. A method as claimed in any one of claims 4 to 7, wherein the chaotrope is selected from the group consisting of guanidine

- 10 -

hydrochloride, guanidine thiocyanate, sodium perchlorate and mixtures thereof.

9. A method as claimed in claim 8, wherein the chaotrope comprises guanidine hydrochloride.

10. A method as claimed in any one of claims 4 to 9, wherein the base comprises a hydroxide.

11. A method as claimed in claim 10, wherein the hydroxide comprises sodium hydroxide.

12. A method as claimed in any one of claims 4 to 11, wherein the organic solvent, the chaotrope, the base and the water are combined to form an extraction mixture, and extraction step (i) comprises mixing the extraction mixture with the plasmid DNA-containing material.

13. A method as claimed in any one of claims 4 to 12, wherein the amount of organic solvent is in the range from 20 to 70% based on the volume of the combination of organic solvent, chaotrope and water.

14. A method as claimed in claim 13, wherein the amount of the organic solvent is in the range from 35 to 50%.

15. A method as claimed in claim 14, wherein the amount of the organic solvent is about 42%.

16. A method as claimed in any one of claims 4 to 15, wherein the chaotrope is present at a concentration of from 0.7M to 1.2M based on the combination of organic solvent, chaotrope and water.

17. A method as claimed in claim 16, wherein the concentration of the chaotrope is about 0.9M.

- 11 -

18. A method as claimed in any one of claims 4 to 17, wherein the recovery step (ii) comprises mixing the DNA-containing organic phase with a precipitating agent that can precipitate the plasmid DNA from the organic solvent, and separating the precipitated plasmid DNA from the solvent.

19. A method as claimed in claim 18, wherein the recovery step (ii) further comprises a washing step in which the precipitated plasmid DNA is washed.

20. A method as claimed in claim 18 or claim 19, wherein the precipitating agent comprises an alcohol.

21. A method as claimed in claim 20, wherein the alcohol comprises ethanol.

22. A method as claimed in any one of claims 18 to 21, wherein the precipitating agent further comprises an acetate salt.

23. A method as claimed in claim 22, wherein the acetate salt comprises sodium acetate.

24. A method as claimed in any one of the preceding claims, which further comprises a step of separating the organic and aqueous phases of step (i) prior to recovering the plasmid DNA.

25. A method as claimed in claim 24, wherein the step of separating the organic and aqueous phases further comprises centrifugation of the mixture formed in step (i) to facilitate separation of the mixture into the organic and aqueous phases.

26. A method as claimed in any one of the preceding claims, wherein the DNA-containing material comprises a lysed or unlysed bacterial culture.

- 12 -

27. An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises a water-immiscible organic solvent capable of supporting plasmid DNA, a chaotrope and water.

28. An extraction mixture as claimed in claim 27, which further comprises a base.

29. An extraction mixture as claimed in claim 28, wherein the base comprises a hydroxide.

30. An extraction mixture as claimed in claim 29, wherein the hydroxide comprises sodium hydroxide.

31. An extraction mixture as claimed in any one of claims 27 to 30, wherein the organic solvent is capable of selectively supporting plasmid DNA to the exclusion of genomic DNA.

32. An extraction mixture as claimed in any one of claims 27 to 31, wherein the organic solvent comprises a C₃ to C₆ alcohol.

33. An extraction mixture as claimed in any one of claims 27 to 32, wherein the organic solvent comprises butanol.

34. An extraction mixture as claimed in claim 33, wherein the butanol comprises n-butanol.

35. An extraction mixture as claimed in any one of claims 27 to 33, wherein the organic solvent constitutes from 20 to 70% based on the volume of the extraction mixture.

36. An extraction mixture as claimed in claim 35, wherein the organic solvent constitutes from 35 to 50 % of the extraction mixture.

- 13 -

37. An extraction mixture as claimed in claim 36, wherein the organic solvent constitutes about 42% of the extraction mixture.

38. An extraction mixture as claimed in any one of claims 27 to 37, wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate and mixtures thereof.

39. An extraction mixture as claimed in claim 38, wherein the chaotrope comprises guanidine hydrochloride.

40. An extraction mixture as claimed in any one of claims 27 to 39, wherein the concentration of chaotrope in the extraction mixture is from 0.7M to 1.2M.

41. An extraction mixture as claimed in claim 40, wherein the concentration of the chaotrope in the extraction mixture is about 0.9M.

AMENDED CLAIMS (Article 34 PCT)

1. A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising the steps of:
 - 5 (i) extracting the plasmid DNA into butanol by mixing the material with butanol, a chaotrope, and water under conditions to denature the genomic DNA; and
 - (ii) recovering the plasmid DNA from the butanol.
2. The method of claim 1, wherein the conditions to denature the DNA comprise basic
10 conditions or a temperature of at least 65°C.
3. The method of claim 2, wherein the conditions to denature the DNA comprise basic conditions in which a base is present.
- 15 4. The method of any preceding claim, wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol.
5. The method of any preceding claim, wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate, and
20 mixtures thereof.
6. The method of claim 6, wherein the chaotrope comprises guanidine hydrochloride.
7. The method of any one of claims 3 to 6, wherein the base comprises a hydroxide.
25
8. The method of claim 7, wherein the hydroxide comprises sodium hydroxide.
9. The method of any one of claims 3 to 8, wherein the butanol, the chaotrope, the base and the water are combined to form an extraction mixture, and extraction step (i) comprises
30 mixing the extraction mixture with the plasmid DNA-containing material.
10. The method of any preceding claim, wherein the amount of butanol is in the range from 20 to 70% based on the volume of the combination of butanol, chaotrope and water.

11. The method of claim 10, wherein the amount of the butanol is in the range from 35 to 50%.
12. The method of claim 11, wherein the amount of the butanol is about 42%.
- 5 13. The method of any preceding claim, wherein the chaotrope is present at a concentration of from 0.7M to 1.2M based on the combination of butanol, chaotrope and water.
14. The method of claim 13, wherein the concentration of the chaotrope is about 0.9M.
- 10 15. The method of any preceding claim, wherein the recovery step (ii) comprises mixing the DNA-containing butanol phase with a precipitating agent that can precipitate the plasmid DNA from the butanol, and separating the precipitated plasmid DNA from the butanol.
16. The method of claim 15, wherein the recovery step (ii) further comprises a washing step
15 in which the precipitated plasmid DNA is washed.
17. The method of claim 15 or claim 16, wherein the precipitating agent comprises an alcohol.
18. The method of claim 17, wherein the alcohol is ethanol.
- 20 19. The method of any one of claims 15 to 18, wherein the precipitating agent further comprises an acetate salt.
20. The method of claim 19, wherein the acetate salt comprises sodium acetate.
- 25 21. The method of any preceding claim, which further comprises a step of separating the organic and aqueous phases of step (i) prior to recovering the plasmid DNA.
22. The method of claim 21, wherein the step of separating the organic and aqueous phases
30 further comprises centrifugation of the mixture formed in step (i) to facilitate separation of the mixture into the organic and aqueous phases.
23. The method of any preceding claim, wherein the DNA-containing material comprises a lysed or unlysed bacterial culture.

24. An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises butanol, a chaotrope, and water.
- 5 25. The extraction mixture of claim 24, which further comprises a base.
26. The extraction mixture of claim 25, wherein the base comprises a hydroxide.
27. The extraction mixture of claim 26, wherein the hydroxide comprises sodium hydroxide.
- 10 28. The extraction mixture of any one of claims 24 to 27, wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol.
29. The extraction mixture of any one of claims 24 to 28, wherein the butanol constitutes from
- 15 20 to 70% based on the volume of the extraction mixture.
30. The extraction mixture of claim 29, wherein the butanol constitutes from 35 to 50 % of the extraction mixture.
- 20 31. The extraction mixture of claim 30, wherein the butanol constitutes about 42% of the extraction mixture.
32. The extraction mixture of any one of claims 24 to 31, wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium
- 25 perchlorate, and mixtures thereof.
33. The extraction mixture of claim 32, wherein the chaotrope comprises guanidine hydrochloride.
- 30 34. The extraction mixture of any one of claims 24 to 33, wherein the concentration of chaotrope in the extraction mixture is from 0.7M to 1.2M.
35. The extraction mixture of claim 34, wherein the concentration of the chaotrope in the extraction mixture is about 0.9M.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☒ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☒ **FADED TEXT OR DRAWING**
- ☒ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINE(S) OR MARK(S) ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.